We claim:

1. An isolated polypeptide comprising a CI-2-like protein with a non-native essential amino acid residue in more than about 11% to less than about 80% of the amino acid residues.
2. The polypeptide of Claim 1 further comprising protein modification.
3. The polypeptide of Claim 1 further comprising from one to 5 disulfide-bonds.
4. The polypeptide of Claim 1 wherein the protein exhibits a free energy of unfolding of more than about 3.5 to less than about 15 Kilocalories per mole.
5. The polypeptide of Claim 1 wherein the polypeptide is proteolytically stable, as demonstrated by detection of the intact polypeptide based upon detection by SDS-PAGE analysis, following a 30 minute incubation at 37°C in 100mM Tris-HCl, 50mMNaCl, 1mM$CaCl_2$, pH 8 , with a 10:1 (weight to weight ratio) of polypeptide:protease, with the protease being either chymotrypsin or trypsin.
6. The polypeptide of Claim 1 further comprising an amino-terminal extension.
7. The polypeptide of Claim 1 wherein the protein exhibits a modified protease activity.
8. The polypeptide of Claim 1 wherein the essential amino acid residues comprise lysine, threonine, tryptophan, methionine or combinations thereof.
9. An isolated polypeptide comprising a plant CI-2-like polypeptide altered to have the following composition: 15-35 mole % lysine, 5-15 mole % methionine, 6-25 mole % threonine, 4-9 mole % tryptophan or combinations thereof.
10. An isolated polypeptide comprising Seq. ID. No. 2, or truncated versions thereof, modified to contain 7 or more non-native essential amino acid residues at positions corresponding to the positions in Sequence ID. No. 2 selected from 1, 8, 11, 17, 18, 19, 20, 22, 23, 31, 34, 38, 40, 41, 47, 49, 56, 58, 59, 60, 61, 62, 63, 65, 67, 69, 73, 75, 76, 78, 79, 81, 82, or combinations thereof.
11. The polypeptide of Claim 10 wherein the essential amino acid residues comprise lysine, threonine, tryptophan, methionine, or combinations or conservative substitutions thereof.

12. The polypeptide of Claim 10 wherein the protein exhibits reduced inhibitory activity against chymotrypsin, subtilisin or elastase.

13. The protein of claim 10 wherein the polypeptide comprises one or more of the following modifications: V32T; E45T; D64T; D74T; or A77T.

14. The protein of claim 10 further comprising one of the following modifications: [T22C, V82C], [E23C, R81C] or [V53C, V70C].

15. The polypeptide of Claim 10 further comprising an amino-terminal extension.

16. The protein according to claim 15 wherein the amino terminal extension comprises a nutritionally-enhancing polypeptide.

17. The polypeptide of Claim 15 wherein the amino-terminal extension is a start signal, a transit sequence, a transit peptide, a signal peptide, a fusion protein, a cleavable peptide, a CI-2-like polypeptide or an uncleaved peptide.

18. The polypeptide of Claim 15 wherein the CI-2 derived polypeptide comprises at least 1 to about 18 additional residues corresponding to amino acid residues 1 to amino acid residue 17 of Seq. ID No. 2 or 12.

19. An isolated polypeptide comprising a CI-2 derived protein comprising two or more of the following modifications corresponding to positions in Seq. ID No. 2 selected from:

H18A, I, L, V or M; N19K or T; L20M I, or V; E23T or K ; S31T or K; E34K or T; V38M I, or L; L40M I, or V; Q41K or T; Q47K or T; I49M I, L, or V; I56K or T; M59G; R62K or T; I63M, L, or V; R65K or T; R67K or T; F69W; L73K or T; A75K or T; Q78K or T; V79T or K; or R81K, or T.

20. The polypeptide according to claim 19 wherein the modifications comprise one or more of the following modifications: [E23C and R81C] or [T22C and V82C] or [V53C and V70C].

21. An isolated polypeptide comprising a CI-2 derived protein comprising two or more of the following modifications corresponding to positions in Seq. ID No. 2 selected from:

H18A or M; N19K; L20M; T22C; E23T or C; S31T; E34K; V38M; L40M; Q41K; Q47K; I49M; I56K; M59G; R62K; I63M; R65K; R67K; F69W; L73K; A75K; Q78K; V79T; R81K or C; or V82C.

22. The polypeptide of claim 21, further comprising substituting a tryptophan at position 61 and a glycine at position 59.

23. The polypeptide of claim 22, further comprising threonine at one or more of positions 32, 45, 53, 64 or 70.

24. The polypeptide according to claim 22 wherein the modifications comprise one or more of the following modifications: [E23C and R81C] or [T22C and V82C] or [V53C and V70C].

25. The polypeptide according to claim 22 further comprising an insert in the active site loop region that is enriched in essential amino acids for the purpose of nutritional enhancement.

26. An isolated polypeptide comprising a CI-2 derived protein comprising two or more of the following modifications corresponding to positions in Seq. ID No. 2 selected from:

[one or more of S1 or S2 or V3 or E4 or K5 or K6 or P7 or E8 or G9 or V10 or N11 or T12 or G13 or A14 or G15 or D16 deleted];

S1K; E8K; N11K; [R17K or M]; [H18A or M]; N19K; L20M; T22 C; [E23T or C]; S31T; V32T; E34K; V38M; L40M; Q41K; E45T; Q47K; I49M; V53C; [[[I56K] and [T58A, or G] and [M59K or G] and [E60A or H] and [Y61W] and [R62K]] or [[I56K] and [M59K or G] and [Y61W] and [R62K]] or [[I56K] and [M59K or G] and [R62K]]];

I63M; D64T; R65K; R67K; F69W; V70C; L73K; D74T; N75K; A77T; Q78K; V79T; [R81K or C]; or V82 C.

27. An isolated polypeptide comprising a CI-2 derived protein comprising modifications corresponding to positions in Seq. ID No. 2 selected from:

[[[I56K] and [T58A, or G] and [M59K or G] and [E60A or H] and [Y61W] and [R62K]] or

[[I56K] and [M59K or G] and [Y61W] and [R62K]] or [[I56K] and [M59K or G] and [R62K]]]; and two or more of the following modifications:

[one or more of S1 or S2 or V3 or E4 or K5 or K6 or P7 or E8 or G9 or V10 or N11 or T12 or G13 or A14 or G15 or D16 deleted];

S1K; E8K; N11K; R17K or M; [H18A or M]; N19K; L20M; T22 C; [E23T or C\; S31T; V32T; E34K; V38M; L40M; Q41K; E45T; Q47K; I49M; V53C;

I63M; D64T; R65K; R67K; F69W; V70C; L73K; D74T; N75K; A77T; Q78K; V79T; [R81K or C]; or V82C.

28. An isolated polypeptide of Sequence ID. No. 2 comprising a protein with three or more non-native essential amino acids at positions selected from 1, 8, 11, 17, 18, 19, 20, 22, 23, 31, 32, 34, 38, 40, 41, 45, 47, 49, 56, 58, 59, 60, 61, 62, 63, 64, 65, 67, 69, 73, 74, 75, 76, 77, 78, 79, 81, 82, or combinations thereof and;

excluding V, P, W, S, E and R at position 56; S, K, R, P, E, V, Y, W, and A at position 58; R, Y, P,W, E, V, S, K, and A at position 59; Q, S,T, I, P, and K at position 60; V, E, R, P, and W at position 61 and E, Q, N, V, F, and Y position 62 and

conservatively modified and conservatively substituted variants thereof.

29. An isolated polypeptide comprising Seq. ID No. 6, 8, 10, 12, 14, 16, 18, 20 or conservatively modified or conservatively substituted variants thereof.

30. An isolated polypeptide comprising at least 23 contiguous amino acids of SEQ. ID Nos. 6, 8, 10, 12, 14, 16, 18, 20.

31. An isolated polypeptide comprising at least 23 contiguous amino acids with more than 79% sequence identity, to the polypeptide of Seq. ID No. 20, wherein the % sequence identity is based on the 23 contiguous amino acids sequence and is determined by GAP analysis using Gap Weight of 12 and Length Weight of 4.

32. An isolated polypeptide that is immunologically reactive with antibodies against the protein of Seq. ID No. 20 and not SEQ ID No. 2.

33. An isolated nucleic acid comprising:

(a) a polynucleotide encoding the protein of claim 1;

(b) a polynucleotide that encodes a polypeptide of SEQ ID NOs: 6, 8, 10, 12, 14, 16, 18, or 20;

(c) a polynucleotide amplified from a plant nucleic acid library using the primers of SEQ ID NOS: 21 and 22;

(d) a polynucleotide comprising at least 20 contiguous bases of SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17 or 19;

(e) a polynucleotide encoding a plant CI-2-derived polypeptide having 15% more essential amino acids than SEQ ID NO 2;

(f) a polynucleotide having at least 73% sequence identity to SEQ ID NO: 19, wherein the % sequence identity is based on the entire sequence and is determined by BLAST 2.0;

(g) a polynucleotide comprising at least 25 nucleotides in length which hybridizes under low stringency conditions to a polynucleotide having the sequence set forth in SEQ ID NOs: 19, wherein the conditions include hybridization with a buffer solution of 30% formamide, 1 M NaCl, 1% SDS at 37°C for 24 hours and a wash in 2X SSC at 50°C, 3x for 15 minutes;

(h) a polynucleotide comprising the sequence set forth in SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17 or 19;

(i) conservatively modified variants of SEQ ID NO : 5, 7, 9, 11, 13, 15, 17 or 19; or

(j) a polynucleotide complementary to a polynucleotide of (a) through (i).

34. The isolated nucleic acid of claim 33 wherein the polynucleotide is a plant polynucleotide.

35. A vector comprising at least one nucleic acid of claim 33.

36. An expression cassette comprising at least one nucleic acid of claim 33 operably linked to a promoter, wherein the nucleic acid is in sense or antisense orientation.

37. A host cell into which is introduced at least one expression cassette of claim 36.

38. The host cell of claim 37 that is a plant cell.

39. A transgenic plant comprising at least one expression cassette of claim 36.

40. The transgenic plant of claim 39, wherein the plant is corn, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, lupin or millet.

41. A seed from the transgenic plant of claim 40.

42. The seed of claim 41, wherein the seed is from corn, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley lupin or millet.

43. A ribonucleic acid sequence encoding a polypeptide of claim 10.

44. A method for increasing the essential amino acid content in a polypeptide in a plant, comprising:

(a) stably transforming a plant cell with the polynucleotide encoding the polypeptide of claim 10, operably linked to a promoter, wherein the polynucleotide is in sense orientation;

(b) growing the plant cell under plant growing conditions to produce a regenerated plant; and

(c) expressing the polypeptide for a time sufficient to produce the polypeptide encoded by the polynucleotide of (a) in the plant.

45. The method of claim 44, wherein the plant cell is corn, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, lupin or millet.

46. A method of increasing expression levels of a protein in a transgenic plant comprising:

engineering a nucleotide sequence encoding the protein of interest to increase the in-vitro proteolytic or thermodynamic stability of the protein; introducing at least 1 copy into a plant cell; and

expressing the protein.

47. A method of increasing nutritional value of feed comprising substituting more than 11% to less than 75% of the amino acids residues of a protein with essential amino acids and modifying the protein to increase the stability of the in-vivo expressed polypeptide.

48. The method of Claim 47 wherein the protein is a CI-2-like polypeptide.

49. The method of Claim 47 wherein the modifying stability is from one or more disulfide bonds.

50. A method of increasing nutritional value of a protein by altering a CI-2 homologue to enhance its nutritional value by altering amino acid residues to the positions in Sequence ID. No. 2 selected from 1, 8, 11, 17, 18, 19, 20, 22, 23, 31, 34, 38, 40, 41, 47, 49, 56, 58, 59, 60, 61, 62, 63, 65, 67, 69, 73, 75, 76, 78, 79, 81, 82, or combinations thereof.

51. An isolated nucleic acid comprising:

(a) a polynucleotide of Seq ID Nos 23, 25, 27, 29 and 31;

(b) a polynucleotide that encodes a polypeptide of SEQ ID NOs: 24, 26, 28, 30, 32;

(c) a polynucleotide amplified from a Zea mays nucleic acid library using the primers of SEQ ID NOS: 21 and 22;

(d) a polynucleotide comprising at least 20 contiguous bases of SEQ ID NOs: 23, 25, 27, 29 and 31;

(e) a polynucleotide having at least 50% sequence identity to SEQ ID NOS: 23, 25, 27, 29 and 31, wherein the % sequence identity is based on the entire sequence and is determined by BLAST 2.0;

(f) a polynucleotide comprising at least 25 nucleotides in length which hybridizes under low stringency conditions to a polynucleotide having the sequence set forth in SEQ ID NOs: 23, 25, 27, 29 and 31, wherein the conditions include hybridization with a buffer solution of 30 % formamide, 1 M NaCl, 1% SDS at 37°C for 4-12 hours and a wash in 2X SSC at 50°C;

(g) a polynucleotide comprising the sequence set forth in SEQ ID NOs: 23, 25, 27, 29 and 31;

(h) conservatively modified variants of SEQ ID NO 23, 25, 27, 29 and 31; or

(i) a polynucleotide complementary to a polynucleotide of (a) through (h).

ABSTRACT OF THE DISCLOSURE

The invention provides isolated nucleic acids and their encoded polypeptides that are involved in enhancing the essential amino acid content of a plant. Optionally there is also a decrease in protease inhibitory activity of the polypeptide. The invention further provides recombinant expression cassettes, host cells, transgenic plants, and antibody compositions. The present invention provides methods and compositions relating to increasing essential amino acid content of plants for feed.